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**BIOFILM DEVELOPMENT IN A
FLUIDIZED BED BIOREACTOR FOR
AEROBIC PHENOL DEGRADATION**

A thesis presented in partial fulfilment
of the requirements for the degree of
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in Biotechnology and Bioprocess Engineering at
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Kirsten Maushake

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*Ki te wao nui a Tane,
ki nga arua a Tangaroa ki uta,
ki nga moana a Tangaroa ki tai.
Ki te ika a Maui,
ki te waka a Maui hoki.*

To the land of the long white cloud.

ABSTRACT

The main objective of this thesis was to follow the biofilm development during start-up of a fluidized bed bioreactor with the help of digital image processing. A mixed microbial culture immobilized on activated carbon particles was grown on phenol as sole carbon source in an aerobic liquid-solid fluidized bed bioreactor. The effect of different reactor temperatures and of different inlet phenol concentrations on the system behaviour during start-up was investigated.

The phenol inhibition kinetics of the culture was studied in batch culture experiments. Three substrate inhibition models (Teissier-Edwards, Haldane and Aiba-Edwards models) were fitted to the experimental data. There was no statistically significant difference in the goodness of fit between the equations. The phenol concentrations at which the fitted functions go through their maximum value were between 57 and 88 mg/l, corresponding to specific growth rates of between 0.64 and 0.65 h⁻¹.

A fluidized bed system was developed and tested. The test runs showed that the most critical part of the apparatus was the liquid distributor at the bottom of the fluidized bed reactor. Other critical factors that were decided on during the test runs were initial bed expansion, flow rate, support particle size, and amount of support particles used, these parameters all being interdependent.

The fluidized bed experiments proved that the use of image analysis techniques is a very effective means of measuring the mean biofilm thickness on fluidized support particles. Micrographs of the bioparticles were analyzed with the help of a software-controlled system. The software identified the circumference of the particle core and the bioparticle. The mean biofilm thickness was calculated

from the projected areas and the perimeters of the bioparticle and the particle core applying a simple trapezoid formula.

In all fluidized bed experiments, the bed stratified into layers (in most cases two or three) containing bioparticles with different biofilm thickness and different biofilm structure. The main focus was on the development of the biofilm in the top layer. The phenol reduction was only small due to a very short hydraulic retention time. Conversely, the dissolved oxygen concentration in the outlet reached very low values. Thus, the system was oxygen-limited.

Different reactor temperatures led to distinct differences in the morphology of the biofilm in the top layer. Without temperature control, i.e. at $\sim 17^{\circ}\text{C}$, and at 30°C , a loose, fluffy, unevenly shaped, thick biofilm developed, whereas at 25°C the biofilm was firm and relatively even in shape, the final thickness remaining far below the values reached by the fluffy biofilm. Since the biofilm that developed at 25°C showed the most favourable characteristics, this temperature was used for the experiments examining the effect of different inlet phenol concentrations.

The biofilm thickness in the top layer increased the fastest at an inlet phenol concentration of 100 mg/l, followed by 35 mg/l, then 330 mg/l and finally 520 mg/l. In the batch culture experiments, the same order had been found for the specific growth rates at phenol concentrations of the above values. In the case of the few observations obtained at non-inhibitory phenol concentrations, the biofilm density increased with increasing phenol concentration. At inhibitory phenol concentrations the flow patterns in the reactor were very different, thus these patterns were the dominating factor influencing the biofilm density.

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NOMENCLATURE

A	projected area of a particle
b	biofilm thickness
CoA	Coenzyme A
d	diameter
d_L	long diameter of an ellipsoid
d_s	short diameter of an ellipsoid
$d_{equiv.}$	equivalent diameter of a sphere
F_{i1}	F-ratio between Model i and Model 1
HRT	hydraulic retention time
i	inhibition constant
K, k_1 , k_2	kinetic constants
K_i	inhibition constant
K_s	saturation constant
m, n	constants
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
P	perimeter
R^2	coefficient of determination
r_s	volumetric substrate uptake rate
S	substrate concentration
S_0	initial substrate concentration
S'	substrate concentration at the onset of the exponential growth phase
S^*	threshold substrate concentration (below which organisms grow apparently without inhibition)
S_m	total inhibition concentration

Std.	standard deviation
x	biomass concentration
$Y_{x/s}$	growth yield coefficient

Greek letters

μ	specific growth rate
μ_m	maximum specific growth rate
μ^*	maximum observable specific growth rate
σ^2	variance

Subscripts

b	bioparticle
c	carrier particle
i	inlet
o	outlet